WEST Search History

DATE: Monday, November 25, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR		
L8	L5 and $carboxamidomethyl	1	L8
L7	"1-(carboxamidomethyl)-dihydronicotinamide"	0	L7
L6	$dihydronicotinamide SAME NRH	0	L6
L5	$dihydronicotinamide	86	L5
L4	L1 and ($dihydronicotinamide or 1-carboxamidomethyl)	0	L4
L3	L2 and $dihydronicotinamide	0	L3
L2	L1 and (prodrug or NRH or NQ$ or CB1954 or CB$)	29	L2
L1	(Knox-R$ or Burke-P$).in.	647	L1

END OF SEARCH HISTORY

(FILE 'HOME' ENTERED AT 10:25:11 ON 25 NOV 2002)

FILE 'MEDLINE, CANCERLIT, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 10:25:19
ON 25 NOV 2002

L1	842	S	?DIHYDRONICOTINAMIDE OR "1-CARBOXAMIDOMETHYL-DIHYDRONICOTINAM
L2	0	S	"1-CARBOXAMIDOMETHYL-DIHYDRONICOTINAMIDE"
L3	24	S	?DIHYDRONICOTINAMIDE AND NRH
L4	7	DUP REM	L3 (17 DUPLICATES REMOVED)
L5	226	S	?CARBOXAMIDOMETHYL
L6	0	S	L5 AND DIHYDRONICOT?
L7	0	S	L5 AND NRH

Connecting via Winsock to STN

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LOGINID:SSSPTA1642GXN

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Apr 08	"Ask CAS" for self-help around the clock
NEWS 3	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4	Apr 09	ZDB will be removed from STN
NEWS 5	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 8	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 9	Jun 03	New e-mail delivery for search results now available
NEWS 10	Jun 10	MEDLINE Reload
NEWS 11	Jun 10	PCTFULL has been reloaded
NEWS 12	Jul 02	FOREGE no longer contains STANDARDS file segment
NEWS 13	Jul 22	USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14	Jul 29	Enhanced polymer searching in REGISTRY
NEWS 15	Jul 30	NETFIRST to be removed from STN
NEWS 16	Aug 08	CANCERLIT reload
NEWS 17	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18	Aug 08	NTIS has been reloaded and enhanced
NEWS 19	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20	Aug 19	IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21	Aug 19	The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 23	Sep 03	JAPIO has been reloaded and enhanced
NEWS 24	Sep 16	Experimental properties added to the REGISTRY file
NEWS 25	Sep 16	Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 26	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 27	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 28	Oct 21	EVENTLINE has been reloaded
NEWS 29	Oct 24	BEILSTEIN adds new search fields
NEWS 30	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 31	Oct 25	MEDLINE SDI run of October 8, 2002
NEWS 32	Nov 18	DKILIT has been renamed APOLLIT

NEWS EXPRESS	October 14 CURRENT WINDOWS VERSION IS V6.01, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

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FILE 'HOME' ENTERED AT 17:21:55 ON 19 NOV 2002

=> file .gary

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 17:22:02 ON 19 NOV 2002

FILE 'CANCERLIT' ENTERED AT 17:22:02 ON 19 NOV 2002

FILE 'BIOSIS' ENTERED AT 17:22:02 ON 19 NOV 2002
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FILE 'SCISEARCH' ENTERED AT 17:22:02 ON 19 NOV 2002
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=> s dinitrophenylaziridine or cb1954
L1 271 DINITROPHENYLAZIRIDINE OR CB1954

=> s dinitrophenylaziridine or cb1954 or cb-1954
L2 619 DINITROPHENYLAZIRIDINE OR CB1954 OR CB-1954

=> s l2 and vivo
L3 123 L2 AND VIVO

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 48 DUP REM L3 (75 DUPLICATES REMOVED)

=> s l4 and human
3 FILES SEARCHED...
L5 26 L4 AND HUMAN

=> s l5 and py<=1997
2 FILES SEARCHED...
4 FILES SEARCHED...
L6 9 L5 AND PY<=1997

=> d ibib abs 1-9

L6 ANSWER 1 OF 9 MEDLINE
ACCESSION NUMBER: 97226858 MEDLINE
DOCUMENT NUMBER: 97226858 PubMed ID: 9081711
TITLE: The expression of bacterial nitroreductase in transgenic mice results in specific cell killing by the prodrug **CB1954**.
COMMENT: Comment in: Gene Ther. 1997 Feb;4(2):80-1
AUTHOR: Drabek D; Guy J; Craig R; Grosveld F
CORPORATE SOURCE: Department of Cell Biology and Genetics, Institute of Cell Biology, Rotterdam, The Netherlands.
SOURCE: GENE THERAPY, **(1997 Feb)** 4 (2) 93-100.

Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970414
Last Updated on STN: 19980206
Entered Medline: 19970403

AB The enzyme nitroreductase, isolated from Escherichia coli B, converts **CB1954** ((5-aziridin-1-yl)-2,4-dinitro-benzamide) into a cytotoxic DNA interstrand cross-linking agent. The E. coli B gene (nfnB, NTR) encoding nitroreductase (NTR) was cloned into eukaryotic expression vectors. When driven by a CMV promoter, 5-10% of the stably transfected mouse fibroblasts expressed the NTR enzyme. These cells were killed at a concentration of 20 microM **CB1954** in comparison to nonexpressing cells which were killed at a much higher concentration (500 microM). We subsequently generated transgenic mice to test the prodrug system in **vivo**. Nitroreductase was expressed specifically in T cells driven by the control elements of the **human** CD2 locus. Upon **CB1954** treatment, transgenic mice show extensive cell depletion in thymus and spleen (14-16% of normal cell numbers), whereas all other tissues are unaffected by prodrug administration. These results raise the possibility of using the NTR gene in anticancer therapy.

L6 ANSWER 2 OF 9 MEDLINE
ACCESSION NUMBER: 93386836 MEDLINE
DOCUMENT NUMBER: 93386836 PubMed ID: 8375021
TITLE: The bioactivation of **CB 1954** and its use as a prodrug in antibody-directed enzyme prodrug therapy (ADEPT).
AUTHOR: Knox R J; Friedlos F; Boland M P
CORPORATE SOURCE: Molecular Pharmacology Unit, Institute of Cancer Research, Sutton, Surrey, United Kingdom.
SOURCE: CANCER AND METASTASIS REVIEWS, **(1993 Jun)** 12 (2) 195-212. Ref: 67
Journal code: 8605731. ISSN: 0891-9992.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199310
ENTRY DATE: Entered STN: 19931105
Last Updated on STN: 19970203
Entered Medline: 19931019

AB Walker cells in **vivo** or in vitro are exceptionally sensitive to the monofunctional alkylating agent **CB 1954** (5-(aziridin-1-yl)-2,4-dinitrobenzamide). The basis of the sensitivity is that **CB 1954** forms DNA interstrand crosslinks in Walker cells but not in insensitive cells. Crosslink formation is due to the aerobic reduction of **CB 1954** to form 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide by the enzyme DT diaphorase. The 4-hydroxylamine can not crosslink DNA directly but requires further activation by a non-enzymatic reaction with a thioester (such as acetyl coenzyme A). As predicted from their measured DT diaphorase activities, a number of rat hepatoma and hepatocyte cell lines are also sensitive to **CB 1954**. However, no **CB 1954**-sensitive tumours or cell lines of **human** origin have been found. This is because the rate of reduction of **CB 1954** by the **human** form of DT diaphorase is much lower than that of the Walker enzyme (ratio of kcat = 6.4). To overcome this intrinsic resistance of **human** cells towards **CB**

1954 a number of strategies have been developed. First, analogues have been developed that are more rapidly reduced by the **human** form of **CB 1954**. Second, the cytotoxicity of **CB 1954** can be potentiated by reduced pyridinium compounds. Third, a **CB 1954** activating enzyme can be targeted to **human** tumours by conjugating it to an antibody (ADEPT). A nitroreductase enzyme has been isolated from E. coli that can bioactivate **CB 1954** much more rapidly than Walker DT diaphorase and is very suitable for ADEPT. Thus **CB 1954** may have a role in the therapy of **human** tumours.

L6 ANSWER 3 OF 9 MEDLINE
ACCESSION NUMBER: 86053846 MEDLINE
DOCUMENT NUMBER: 86053846 PubMed ID: 3940225
TITLE: **CB 1954** revisited. II. Toxicity and antitumour activity.
AUTHOR: Workman P; Morgan J E; Talbot K; Wright K A; Donaldson J; Twentyman P R
SOURCE: CANCER CHEMOTHERAPY AND PHARMACOLOGY, **(1986)** 16 (1) 9-14.
Journal code: 7806519. ISSN: 0344-5704.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198601
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860122

AB We have assessed the antitumour activity of the nitrophenylaziridine **CB 1954** in vitro and in **vivo**. For EMT6 mouse mammary tumour multicellular spheroids under hypoxic conditions in vitro, a 6-h exposure to 40 micrograms/ml reduced the surviving fraction to as low as 10(-3) and the growth delay was 5.4 days. Oxic cells were twofold less sensitive. Phenyl AIC protected oxic and hypoxic cells equally. Under oxic conditions minimal cell killing was seen with HT29 cells, either in multicellular spheroids or in monolayer; a 6-h exposure to 40 micrograms/ml gave a spheroid growth delay of 1.5-1.7 days. No growth delay was seen with single maximum tolerated doses of **CB 1954** against HT29 grown as a xenograft in immunosuppressed mice. Only minimal growth delays of 1-2 days were seen with similar doses against the EMT6 tumour and the RIF-1 and KHT sarcomas in mice. Little activity was seen with maximum tolerated doses given once a day for 5 days against EMT6 and RIF-1. No chemosensitization was measurable with CCNU, cyclophosphamide or melphalan in the KHT tumour.

L6 ANSWER 4 OF 9 CANCERLIT
ACCESSION NUMBER: 89649497 CANCERLIT
DOCUMENT NUMBER: 89649497
TITLE: CONCEPTS AND MECHANISMS IN HYPOXIC CELL SENSITIZATION.
AUTHOR: Anonymous
CORPORATE SOURCE: No affiliation given.
SOURCE: Non-serial, **(1988)** Sixth Conference on Chemical Modifiers of Cancer Treatment. March 21-25, 1988, Paris, Alpha Therapeutics, I. Hoffman La Roche and Co., E.I. Dupont Nemours and Co., p. 2.1-2.30, 1988. .
DOCUMENT TYPE: Book; (MONOGRAPH)
LANGUAGE: English
FILE SEGMENT: Institute for Cell and Developmental Biology
ENTRY MONTH: 198902
ENTRY DATE: Entered STN: 19941107
Last Updated on STN: 19970509

AB A session on concepts and mechanisms in hypoxic cell sensitization, included in the Sixth Conference on Chemical Modifiers of Cancer

Treatment, held in Paris, France, March 21-25, 1988, consisted of the following papers: comparative DNA damage and repair induced by misonidazole, **CB-1954**, and RSU-1069; induction of SOS repair of DNA by misonidazole, **CB-1954**, and RSU-1069; the reaction between nitracrine and glutathione: implications for hypoxic cell radiosensitization and cytotoxicity; radiosensitizer-DNA interactions in relation to intracellular and intranuclear uptake; reactions of 1-methyl-2-nitrosoimidazole and glutathione; effects of single and multiple doses of misonidazole on intermediary metabolism in normal mouse tissues; accelerated elimination of pimonidazole following microsomal enzyme induction in mice: a possible approach to reduced neurotoxicity of the pimonidazole-etanidazole combination; 1-methyl-2-nitrosoimidazole: cytotoxic and glutathione depleting capabilities; evaluation of nitroimidazole hypoxic cell radiosensitizers in a **human** tumor cell line high in intracellular glutathione; inhibition of polyamine biosynthesis and hypoxic cell radiosensitization in **human** lung carcinoma cells; the cytotoxic and radiosensitizing effect of misonidazole in four mammalian cell lines; combined radiation-protective and radiation-sensitizing agents IV: measurement of intracellular protector concentrations; RB-6145--a promising analog of the dual function radiation sensitizer RSU-1069; RA-263: a nitroimidazole as effective as misonidazole; enhanced response of polyamine depletion in radiosensitization of hypoxic cells with a putrescine analog of 2-nitroimidazole; KIH-802: 2-nitroimidazole-1-acetohydroxamate as a 'post-misonidazole' hypoxic cell radiosensitizer; radiosensitizing effect of new nitroimidazole derivatives to murine tumors; importance of tumor affinity of nitroazoles in hypoxic radiosensitization; MST-02, a potent radiosensitizer; NLP-1: a DNA intercalating hypoxic cell radiosensitizer and cytotoxin; potential hypoxic cell sensitizers: nucleoside analogs III; characteristics of fluorinated nitroazoles as hypoxic cell radiosensitizers; radiation sensitization of hypoxic cells by a new compound: N-(3-nitro-4-quinolyl)-morpholino-carboxamidine; biological response and chemical properties of a series of nitrothiophene derivatives; the study of the mechanisms of an extract from Chinese herbal medicine '764-1' as a radiosensitizer and its effect on cancer metastasis; Cu(II) complexes as possible modifiers of radiation effects; potentiation of radiation-induced cell kill by synthetic metalloporphyrins; high efficiency of ferricenium salts as radiosensitizers in vitro and in **vivo**; chemical and biological studies of the tautomeric forms of 4(5)-nitroimidazole stabilized on platinum; and effects of halide and sulfoxide replacements on radiosensitizing properties of Ru-nitroimidazole complexes.

L6 ANSWER 5 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:425819 BIOSIS
DOCUMENT NUMBER: PREV199799725022
TITLE: Gene therapy for pancreatic cancer: In **vivo** killing of tumour cells expressing E. coli nitroreductase following administration of the prodrug **CB1954**.
AUTHOR(S): Green, N. K. (1); Youngs, D. J. (1); Kerr, D. J.; Neoptolemos, J. P.; Searle, P. F.
CORPORATE SOURCE: (1) Dep. Surg., Univ. Birmingham, Birmingham UK
SOURCE: Digestion, (1997) Vol. 58, No. SUPPL. 2, pp. 6.
Meeting Info.: 29th European Pancreatic Club Meeting London, England, UK July 9-12, 1997
ISSN: 0012-2823.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L6 ANSWER 6 OF 9 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97158391 EMBASE
DOCUMENT NUMBER: 1997158391
TITLE: Selective cell ablation in the mammary gland of transgenic mice.

AUTHOR: Gusterson B.; Cui W.; Iwobi M.; Crompton M.R.; Harold G.; Hobbs S.; Kamalati T.; Knox R.; Neil C.; Yull F.; Howard B.; Clark A.J.

CORPORATE SOURCE: B. Gusterson, Cell Biol./Experimental Pathol. Sec., Haddow Laboratories, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, United Kingdom

SOURCE: Endocrine-Related Cancer, (1997) 4/1 (67-74).
Refs: 39
ISSN: 1351-0088 CODEN: ERCAE

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 016 Cancer
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We have generated transgenic mice which express the gene encoding E. coli nitro-reductase (NTR) specifically in the luminal epithelial cells of the mammary gland and shown that administration of the anti-tumour drug **CB1954** rapidly and selectively kills these cells. Owing to the ease of control of NTR-mediated cell ablation, we anticipate that this system will supercede herpes simplex virus type 1 thymidine kinase. There are widespread potential applications for this approach in the dissection of complex cellular interactions during development and in the adult organism. The present transgenic model also has important applications for the study in **vivo** of novel prodrugs that can be selected for variable degrees of bystander effects. Such studies will have particular significance for those groups advocating the use of NTR as an appropriate enzyme for gene-directed enzyme prodrug therapy by providing models of a wide range of **human** disease for mechanistic and therapeutic experimentation. The model described in this work has potential for the examination of mammary carcinogenesis and its modulation through manipulation of the size of the target cell populations.

L6 ANSWER 7 OF 9 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 76193287 EMBASE

DOCUMENT NUMBER: 1976193287

TITLE: Screening for anti cancer agents; the relative merits of in vitro and in **vivo** techniques.

AUTHOR: Connors T.A.; Phillips B.J.

CORPORATE SOURCE: Chester Beatty Res. Inst., Roy. Cancer Hosp., London, United Kingdom

SOURCE: Biochemical Pharmacology, (1975) 24/24 (2217-2224).
CODEN: BCPCA6

DOCUMENT TYPE: Journal

FILE SEGMENT: 037 Drug Literature Index
030 Pharmacology
016 Cancer

LANGUAGE: English

AB The most successful systems thus far have been methods involving transplanted animal tumors. It has been proposed that anti cancer agents will only be found by using **human** cancers as the test system. The most promising systems involve **human** cancers being transplanted into immunologically dependent mice. The various methods which are discussed in this paper includes the testing of various chemotherapeutic agents in vitro and in **vivo**. The special problems in each system includes cell selection and cell kinetics, anti tumor selectivity, tumor host relationships and the biotransformation of the drugs used. Another important aspect of this problem is the heterotransplantation of **human** tumors. (Calesnick - Springfield, Pa).

L6 ANSWER 8 OF 9 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 92:471838 SCISEARCH

THE GENUINE ARTICLE: JG580

TITLE: DT-DIAPHORASE ACTIVITY CORRELATES WITH SENSITIVITY TO THE INDOLOQUINONE-EO9 IN MOUSE AND **HUMAN** COLON CARCINOMAS

AUTHOR: WALTON M I; BIBBY M C; DOUBLE J A; PLUMB J A; WORKMAN P (Reprint)

CORPORATE SOURCE: UNIV GLASGOW, CANC RES CAMPAIGN, DEPT MED ONCOL, BEATSON LABS, SWITCHBACK RD, GLASGOW G61 1BD, SCOTLAND; UNIV CAMBRIDGE, CTR MRC, MRC, CLIN ONCOL & RADIOTHERAPEUT UNIT, CAMBRIDGE CB2 2QH, ENGLAND; UNIV BRADFORD, CLIN ONCOL UNIT, BRADFORD BD7 1DP, W YORKSHIRE, ENGLAND

COUNTRY OF AUTHOR: SCOTLAND; ENGLAND

SOURCE: EUROPEAN JOURNAL OF CANCER, (**1992**) Vol. 28A, No. 10, pp. 1597-1600.
ISSN: 0964-1947.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The indoloquinone EO9 exhibits promising in vitro and in **vivo** antitumour activity. EO9 is metabolised to DNA damaging species by DT-diaphorase in vitro. In the present study DT-diaphorase specific activity was 16 fold higher in the mouse adenocarcinoma MAC 16, a tumour which is quite responsive to EO9 in **vivo**, compared with levels in the more resistant mouse adenocarcinoma MAC 26. This order of responsiveness is the reverse of that seen with the most active of the clinically used agents in these tumours [chloroethylnitrosoureas and 5-fluorouracil (5-FU)]. In addition, when the in vitro sensitivity of two **human** colon carcinoma cell lines was compared, EO9 was 15-30 fold more active in the DT-diaphorase rich HT29 line than in the enzyme-deficient BE cell line counterpart. These results are consistent with the hypothesis that DT-diaphorase expression may be a major determinant of the sensitivity of tumours to EO9. This should be considered in the clinical development of the drug.

L6 ANSWER 9 OF 9 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 91:625277 SCISEARCH

THE GENUINE ARTICLE: GP085

TITLE: THE WALKER-256 CARCINOMA - A CELL TYPE INHERENTLY SENSITIVE ONLY TO THOSE DIFUNCTIONAL AGENTS THAT CAN FORM DNA INTERSTRAND CROSS-LINKS

AUTHOR: KNOX R J (Reprint); LYDALL D A; FRIEDLOS F; BASHAM C; RAWLINGS C J; ROBERTS J J

CORPORATE SOURCE: INST CANC RES, MOLEC PHARMACOL UNIT, DRUG DEV SECT, COTSWOLD RD, SUTTON SM2 5NG, SURREY, ENGLAND (Reprint)

COUNTRY OF AUTHOR: ENGLAND

SOURCE: MUTATION RESEARCH, (**1991**) Vol. 255, No. 3, pp. 227-240.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 46

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Walker 256 rat tumour has been maintained in **vivo** for over 60 years and until recently was used as a primary screen for new antitumour agents. This screen was particularly useful in identifying difunctional alkylating agents as potentially useful anticancer agents and it would seem that the Walker tumour is composed of cells sensitive towards this type of agent.

A cell line (WS) established from the Walker tumour retained the sensitivity of the tumour towards difunctional agents and we have examined its phenotype in comparision to a derived, resistant, cell line (WR). The response of WR cells to a range of cytotoxic agents was similar to other established cell lines whilst WS cells were much more sensitive only

towards difunctional reacting agents. There were no significant differences in the binding of these agents to the DNA of WS or WR cells. All the agents towards which WS cells showed sensitivity were, without exception, capable of reacting with DNA in Walker cells and forming DNA-DNA interstrand crosslinks. WS cells were not sensitive to busulphan, BCNU, CCNU or Me-CCNU but these agents did not produce interstrand crosslinks in the DNA of either WS or WR cells. Thus WS cells are intrinsically sensitive to specific DNA damage and this is probably a DNA interstrand crosslink.

Hybrid cells produced by fusion of WS with WR cells lacked the inherent sensitivity of the WS cells towards cisplatin; sensitivity was therefore a recessive characteristic. Transfection of WS cells with **human** DNA also gave rise to 2 cisplatin-resistant clones, although it could not be ascertained if these clones were true transfectants or revertants. The survival of these resistant clones, after treatment with cisplatin, was about the same as WR cells a finding which would be consistent with complementation by a transferred gene or reversion of a single gene defect in WS cells. In their sensitivity only to difunctional compounds and lack of an apparent DNA excision repair defect the phenotype of Walker cells strongly resembles those cells from **human** patients suffering from Fanconi's anaemia and also of yeast snm1 mutant cells. The mechanisms giving rise to this failure to tolerate specific DNA damage (which seems to involve the inability to recover from the initial inhibition of DNA synthesis and may involve a single defect of a gene involved in the late steps of crosslink repair), do not involve drug uptake, drug binding to DNA, cell size, cell doubling time or DNA excision repair.

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=> d his

     (FILE 'HOME' ENTERED AT 17:21:55 ON 19 NOV 2002)

     FILE 'MEDLINE, CANCERLIT, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 17:22:02
     ON 19 NOV 2002
L1          271 S DINITROPHENYLAZIRIDINE OR CB1954
L2          619 S DINITROPHENYLAZIRIDINE OR CB1954 OR CB-1954
L3          123 S L2 AND VIVO
L4           48 DUP REM L3 (75 DUPLICATES REMOVED)
L5           26 S L4 AND HUMAN
L6            9 S L5 AND PY<=1997

=> s l2 and (reduc? or nicot?)
L7          273 L2 AND (REDUC? OR NICOT?)

=> s l7 and py<=1997
   2 FILES SEARCHED...
   3 FILES SEARCHED...
L8          167 L7 AND PY<=1997

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9           68 DUP REM L8 (99 DUPLICATES REMOVED)

=> s l9 and human
   4 FILES SEARCHED...
L10          26 L9 AND HUMAN

=> d his

     (FILE 'HOME' ENTERED AT 17:21:55 ON 19 NOV 2002)

     FILE 'MEDLINE, CANCERLIT, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 17:22:02
     ON 19 NOV 2002
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L1          271 S DINITROPHENYLAZIRIDINE OR CB1954
L2          619 S DINITROPHENYLAZIRIDINE OR CB1954 OR CB-1954
L3          123 S L2 AND VIVO
L4           48 DUP REM L3 (75 DUPLICATES REMOVED)
L5           26 S L4 AND HUMAN
L6            9 S L5 AND PY<=1997
L7          273 S L2 AND (REDUC? OR NICOT?)
L8          167 S L7 AND PY<=1997
L9           68 DUP REM L8 (99 DUPLICATES REMOVED)
L10          26 S L9 AND HUMAN

=> s l2 and nicot?
L11          73 L2 AND NICOT?

=> s l11 and human
   4 FILES SEARCHED...
L12          47 L11 AND HUMAN

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13          28 DUP REM L12 (19 DUPLICATES REMOVED)

=> s l13 and py<=1997
   2 FILES SEARCHED...
   3 FILES SEARCHED...
L14          17 L13 AND PY<=1997

=> d ibib abs 1-17

L14  ANSWER 1 OF 17     MEDLINE
ACCESSION NUMBER:   97153088     MEDLINE
DOCUMENT NUMBER:    97153088   PubMed ID: 8999809
TITLE:              Molecular basis of the catalytic differences among
                    DT-diaphorase of human, rat, and mouse.
AUTHOR:             Chen S; Knox R; Wu K; Deng P S; Zhou D; Bianchet M A; Amzel
                    L M
CORPORATE SOURCE:   Division of Immunology, Beckman Research Institute of the
                    City of Hope, Duarte, California 91010, USA..
                    schen@smptplink.coh.org
CONTRACT NUMBER:    GM45540 (NIGMS)
SOURCE:             JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jan 17)
                    272 (3) 1437-9.
                    Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        199702
ENTRY DATE:         Entered STN: 19970227
                    Last Updated on STN: 19970227
                    Entered Medline: 19970213
AB   DT-diaphorase (EC 1.6.99.2), also referred to as NAD(P)H:(quinone-
     acceptor) oxidoreductase, is involved in the reductive activation process
     of several cytotoxic antitumor quinones and nitrobenzenes. It has been
     observed in our and other laboratories that the rat enzyme is
     significantly more effective in activating these drugs than the
     human and mouse enzymes. These results indicate that the available
     cytotoxic drugs are better substrates for the rat enzyme and are not the
     most ideal prodrugs for activation by DT-diaphorase in human
     tumors. In this study, using site-directed mutagenesis to replace residues
     in the rat enzyme with the human sequences and residues in the
     human enzyme with the rat sequences, we have found that residue
     104 (Tyr in the rat enzyme and Gln in the human and mouse
     enzymes) is an important residue responsible for the catalytic differences
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between the rat and the **human** (and mouse) enzymes. With an exchange of a single amino acid, the rat mutant Y104Q behaved like the wild-type **human** enzyme, and the **human** mutant Q104Y behaved like the wild-type rat enzyme in their ability to reductively activate the cytotoxic drug **CB 1954** (5-(aziridin-1-yl)-2,4-dinitrobenzamide). The study also confirms the conclusion of the x-ray structural analysis of rat enzyme that residue 130 (Thr in the rat enzyme and Ala in the **human** and mouse enzymes) is positioned near the binding region of the **nicotinamide** portion of NAD(P)H. This structural information is very important for designing suitable drugs and approaches for **human** cancer chemotherapy mediated by DT-diaphorase.

L14 ANSWER 2 OF 17 MEDLINE
ACCESSION NUMBER: 93080655 MEDLINE
DOCUMENT NUMBER: 93080655 PubMed ID: 1449531
TITLE: Potentiation of **CB 1954** cytotoxicity by reduced pyridine nucleotides in **human** tumour cells by stimulation of DT diaphorase activity.
AUTHOR: Friedlos F; Biggs P J; Abrahamson J A; Knox R J
CORPORATE SOURCE: Molecular Pharmacology Unit, Institute of Cancer Research, Sutton, Surrey, U.K.
SOURCE: BIOCHEMICAL PHARMACOLOGY, **(1992 Nov 3)** 44 (9) 1739-43.
Journal code: 0101032. ISSN: 0006-2952.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19970203
Entered Medline: 19921230

AB The toxicity of **CB 1954** [5-(aziridin-1-yl)-2,4-dinitrobenzamide] towards **human** cells was greatly enhanced by NADH (when foetal calf serum was present in the culture medium) and by **nicotinamide** riboside (reduced) (NRH), but not by **nicotinate** riboside (reduced). Co-treatment of **human** cells with **CB 1954** and NADH resulted in the formation of crosslinks in their DNA. The toxicity produced by other DNA crosslinking agents was unaffected by reduced **nicotinamide** compounds. When caffeine was included in the medium, a reduction in the cytotoxicity of **CB 1954** occurred. The toxicity experienced by **human** cell lines after exposure to **CB 1954** and NADH was proportional to their levels of the enzyme DT diaphorase NAD(P)H dehydrogenase (quinone), EC 1.6.99.2. It is concluded that NRH, which we have shown to be a co-factor for rat DT diaphorase (Friedlos et al., Biochem Pharmacol 44: 25-31, 1992), is generated from NADH by enzymes in foetal calf serum, and stimulates the activity of **human** DT diaphorase towards **CB 1954**.

L14 ANSWER 3 OF 17 MEDLINE
ACCESSION NUMBER: 92378681 MEDLINE
DOCUMENT NUMBER: 92378681 PubMed ID: 1387314
TITLE: Metabolism of NAD(P)H by blood components. Relevance to bioreductively activated prodrugs in a targeted enzyme therapy system.
AUTHOR: Friedlos F; Knox R J
CORPORATE SOURCE: Molecular Pharmacology Unit, Institute of Cancer Research, Surrey, U.K.
SOURCE: BIOCHEMICAL PHARMACOLOGY, **(1992 Aug 18)** 44 (4) 631-5.
Journal code: 0101032. ISSN: 0006-2952.
PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19921009
Last Updated on STN: 19970203
Entered Medline: 19920921

AB NADH was metabolized both by serum components and at the cell surface. The metabolism by serum was either oxidation to NAD+, or hydrolysis of the pyrophosphate to yield **nicotinamide** mononucleotide (reduced) (NMNH) and AMP. NMNH was further hydrolysed to yield **nicotinamide** riboside (reduced) (NRH), which was stable. NAD+ was hydrolysed (although at a slower rate than was NADH), but was also reduced to yield NADH. The reduction of NAD+ was catalysed by the enzyme serum L(+)lactate dehydrogenase (EC 1.1.1.27) and was dependent on the concentration of L(+)lactate in the serum. NADPH was hydrolysed in a similar manner to NADH but not oxidized by serum. NADH generated from NAD+ by serum derived from **human**, foetal calf and horse sources was capable of driving the bioreductive activation of **CB 1954** by the enzyme DT diaphorase. Cell surfaces oxidized NADH to NAD+, but did not oxidize NADPH or NRH. These observations suggest that NAD(P)H would be unsuitable as a source of reducing equivalents for the bioreductive activation of prodrugs by a reductase enzyme in Antibody Directed Enzyme Prodrug Therapy (ADEPT). In contrast, NAD+ (which could act as a source of NADH) and NRH could avoid the shortcomings of NAD(P)H, and act as suitable cofactors for an enzyme in an ADEPT system.

L14 ANSWER 4 OF 17 CANCERLIT
ACCESSION NUMBER: 1998640756 CANCERLIT
DOCUMENT NUMBER: 98640756
TITLE: Molecular basis of the catalytic differences among DT-diaphorase of **human**, rat, and mouse (Meeting abstract).
AUTHOR: Anonymous
CORPORATE SOURCE: Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010.
SOURCE: Proc Annu Meet Am Assoc Cancer Res, **(1997)** 38 A3756.
ISSN: 0197-016X.
DOCUMENT TYPE: (MEETING ABSTRACTS)
LANGUAGE: English
FILE SEGMENT: Institute for Cell and Developmental Biology
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980805
Last Updated on STN: 19980805

AB DT-Diaphorase (EC 1.6.99.2), also referred to as NAD(P)H: quinone acceptor oxidoreductase, is involved in the reductive activation process of several cytotoxic anti-tumor quinones and nitrobenzenes. It has been observed in our and other laboratories that the rat enzyme is significantly more effective in activating these drugs than the **human** and mouse enzymes. These results indicate that the available cytotoxic drugs are better substrates for the rat enzyme and are not the most ideal prodrugs for activation by DT-diaphorase in **human** tumors. In this study, using site-directed mutagenesis to replace residues in the rat enzyme with the **human** sequences and residues in the **human** enzyme with the rat sequences, we have found that residue-104 (Tyr in the rat enzyme and Gln in the **human** and mouse enzymes) is an important residue responsible for the catalytic differences between the rat and the **human** (and mouse) enzymes. With an exchange of a single amino acid, the rat mutant Y104Q behaved like the wild-type **human** enzyme, and the **human** mutant Q104Y behaved like the wild-type rat enzyme in their ability to reductively activate the cytotoxic drug **CB1954** [5-(aziridin-1-yl)-2,4-dinitrobenzamide]. The study also confirms the conclusion of the x-ray structural analysis of rat enzyme

that residue-130 (Thr in the rat enzyme and Ala in the **human** and mouse enzymes) is positioned near the binding region of the **nicotinamide** portion of NAD(P)H. This structural information is very important for designing suitable drugs and approaches for **human** cancer chemotherapy mediated by DT-diaphorase.

L14 ANSWER 5 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97356093 EMBASE
DOCUMENT NUMBER: 1997356093
TITLE: Catalytic properties of NAD(P)H:quinone oxidoreductase-2 (NQO2), a dihydronicotinamide riboside dependent oxidoreductase.
AUTHOR: Wu K.; Knox R.; Xiu Zhu Sun; Joseph P.; Jaiswal A.K.; Zhang D.; Deng P.S.- K.; Chen S.
CORPORATE SOURCE: S. Chen, Division of Immunology, City of Hope Beckman Research Inst., Duarte, CA 91010, United States. schen@smtplink.coh.org
SOURCE: Archives of Biochemistry and Biophysics, (1997) 347/2 (221-228).
Refs: 27
ISSN: 0003-9861 CODEN: ABBIA4
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Human** NAD(P)H:quinone acceptor oxidoreductase-2 (NQO2) has been prepared using an Escherichia coli expression method NQO2 is thought to be an isoform of DT-diaphorase (EC 1.6.99.2) [also referred to as NAD(P)H:quinone acceptor oxidoreductase] because there is a 49% identity between their amino acid sequences. The present investigation has revealed that like DT-diaphorase, NQO2 is a dimer enzyme with one FAD prosthetic group per subunit. Interestingly, NQO2 uses dihydronicotinamide riboside (NRH) rather than NAD(P)H as an electron donor. It catalyzes a two-electron reduction of quinones and oxidation-reduction dyes. One-electron acceptors, such as potassium ferricyanide, cannot be reduced by NQO2. This enzyme also catalyzes a four-electron reduction, using methyl red as the electron acceptor. The NRH-methyl red reductase activity of NQO2 is 11 times the NADH-methyl red reductase activity of DT-diaphorase. In addition, through a four-electron reduction reaction, NQO2 can catalyze nitroreduction of cytotoxic compound **CB 1954** [5-(aziridin-1-yl)-2,4-dinitrobenzamide]. NQ02 is 3000 times more effective than DT-diaphorase in the reduction of **CB 1954**. Therefore, NQO2 is a NRH-dependent oxidoreductase which catalyzes two- and four-electron reduction reactions, NQO2 is resistant to typical inhibitors of DT- diaphorase, such as dicumarol, Cibacron blue, and phenindone. Flavones are inhibitors of NQO2. However, structural requirements of flavones for the inhibition of NQO2 are different from those for DT-diaphorase. The most potent flavone inhibitor tested so far is quercetin (3,5,7,3',4'- .6pentahydroxyflavone). It has been found that quercetin is a competitive inhibitor with respect to NRH (K1 = 21 nM). NQO2 is 43 amino acids shorter than DT-diaphorase, and it has been suggested that the carboxyl terminus of DT-diaphorase plays a role in substrate binding (S. Chen et al., Protein Sci. 3, 51-57, 1994). In order to understand better the basis of catalytic differences between NQO2 and DT-diaphorase, a **human** NQO2 with 43 amino acids from the carboxyl terminus of **human** DT-diaphorase (i.e., hNQO2-hDT43) has been prepared. hNQO2-hDT43 still uses NRH as an electron donor. In addition, the chimeric enzyme is inhibited by quercetin but not dicumarol. These results suggest that additional region(s) in these enzymes is involved in differentiating NRH from NAD(P)H.

L14 ANSWER 6 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97066955 EMBASE

DOCUMENT NUMBER: 1997066955
TITLE: **Human** DT-diaphorase as a candidate for enzyme-directed bioreductive drug development.
AUTHOR: Phillips R.M.
CORPORATE SOURCE: R.M. Phillips, Clinical Oncology Unit, University of Bradford, Bradford BD7 1DP, United Kingdom
SOURCE: Drugs of the Future, (1996) 21/12 (1247-1256).
Refs: 130
ISSN: 0377-8282 CODEN: DRFUD4
COUNTRY: Spain
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English

L14 ANSWER 7 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96215853 EMBASE
DOCUMENT NUMBER: 1996215853
TITLE: Enzymology of bioreductive drug activation.
AUTHOR: Ross D.; Beall H.D.; Siegel D.; Traver R.D.; Gustafson D.L.
CORPORATE SOURCE: School of Pharmacy and Cancer Center, University of Colorado, Health Sciences Center, 4200 E Ninth Avenue,Denver, CO 80262, United States
SOURCE: British Journal of Cancer, (1996) 74/SUPPL. XXVII (S1-S8).
ISSN: 0007-0920 CODEN: BJCAAI
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English

L14 ANSWER 8 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95150353 EMBASE
DOCUMENT NUMBER: 1995150353
TITLE: Catalytic properties of NAD(P)H:quinone acceptor oxidoreductase: Study involving mouse, rat, **human** , and mouse-rat chimeric enzymes.
AUTHOR: Chen S.; Knox R.; Lewis A.D.; Friedlos F.; Workman P.; Deng P.S.K.; Fung M.; Ebenstein D.; Wu K.; Tsai T.-M.
CORPORATE SOURCE: Division of Immunology, City of Hope Beckman Res. Institute, 1450 E. Duarte Rd.,Duarte, CA 91010, United States
SOURCE: Molecular Pharmacology, (1995) 47/5 (934-939).
ISSN: 0026-895X CODEN: MOPMA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB NAD(P)H:quinone acceptor oxidoreductase (quinone reductase) (DT-diaphorase, EC 1.6.99.2) is involved in the process of reductive activation of cytotoxic antitumor quinones and nitrobenzenes. In this study, we initially examined the relative abilities of mouse, rat, and **human** quinone reductases to reduce two prodrugs, **CB 1954** [5-(aziridin-1-yl)-2,4- dinitrobenzamide] and EO9 [5-(1-aziridinyl)-3-(hydroxymethyl)-2-(3-hydroxy- 1-propenyl)-1-methyl-1H-indole-4,7-dione]. By using Escherichia coli- expressed quinone reductases

and evaluating them under identical conditions, we confirmed previous findings showing that the **human** enzyme is not as effective as the rat enzyme in reducing **CB 1954** and EO9, although the two enzymes have similar NAD(P)H-menadione reductase activities. Interestingly, although the amino acid sequence of mouse quinone reductase is more homologous to that of the rat enzyme, we found that the mouse enzyme behaves similarly to the **human** enzyme in its ability to reduce these compounds and to generate drug-induced DNA damage. To determine the region of quinone reductase that is responsible for the catalytic differences, two mouse-rat chimeric enzymes were generated. MR-P, a chimeric enzyme that has mouse amino-terminal and rat carboxyl-terminal segments of quinone reductase, was shown to have catalytic properties resembling those of rat quinone reductase, and RM-P, a chimeric enzyme that has rat amino-terminal and mouse carboxyl- terminal segments of quinone reductase, was shown to have catalytic properties resembling those of mouse quinone reductase. In addition, MR-P and RM-P were found to be inhibited by flavones with K(i) values similar to those for rat and mouse quinone reductases, respectively. Based on these results, we propose that the carboxyl-terminal portion of the enzyme plays an important role in the reduction of cytotoxic drugs and the binding of flavones.

L14 ANSWER 9 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95101049 EMBASE
DOCUMENT NUMBER: 1995101049
TITLE: Bioactivation of quinones by DT-diaphorase, molecular, biochemical, and chemical studies.
AUTHOR: Ross D.; Beall H.; Traver R.D.; Siegel D.; Phillips R.M.; Gibson N.W.
CORPORATE SOURCE: School of Pharmacy and Cancer Center, Colorado Univ. Health Sciences Ctr., 4200 East 9th Avenue,Denver, CO 80262, United States
SOURCE: Oncology Research, (1994) 6/10-11 (493-500).
ISSN: 0965-0407 CODEN: ONREE8
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Because of the elevated DT-diaphorase (DTD) activity in certain tumors such as **human** nonsmall cell lung cancer (NCSLC), DTD is a potential target on which to base the development of new antitumor compounds. Mitomycin C is the most effective single agent used for the therapy of NSCLC and is metabolized and bioactivated by DTD. Mitomycin C is a poor substrate for DTD, however, and its metabolism is pH-dependent. We have therefore focused on identifying more efficient substrates for DTD. We have developed a metabolic and cytotoxicity screen that identifies compounds which are efficiently bioactivated by DTD. This screen utilizes both aerobic and hypoxic conditions and cell lines with both elevated and deficient DTD activity as an index of selectivity. Using the screen described above, we have identified [3-hydroxy- 5-aziridinyl-1-methyl-2-(1H-indole-4,7-indione)-prop-β-en-α-ol] (E09), 2,5-diaziridinyl-1,4-benzoquinone (MeDZQ), and streptonigrin as compounds that are most efficiently bioactivated by DTD and exert selective cytotoxicity. Although certain tumors such as NSCLC have elevated DTD activity, we have characterized a point mutation at position 609 in the DTD cDNA, which codes for a proline to serine change in the protein and leads to a loss of enzyme activity. We have characterized this mutation in both BE **human** colon carcinoma cells and H596 **human** NSCLC cells. This mutation and resulting lack of DTD activity complicates the use of

agents designed to target DTD in tumors. An enzyme-directed approach to chemotherapy utilizing DTD as a target is still a viable strategy, however, providing that pretreatment biopsies can be obtained and screened for DTD activity.

L14 ANSWER 10 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95101046 EMBASE
DOCUMENT NUMBER: 1995101046
TITLE: Enzyme-directed bioreductive drug development revisited: A commentary on recent progress and future prospects with emphasis on quinone anticancer agents and quinone metabolizing enzymes, particularly DT-diaphorase.
AUTHOR: Workman P.
CORPORATE SOURCE: Cancer Research Department, ZENECA Pharmaceuticals, Alderley Park,Macclesfield, Cheshire SK10 4TG, United Kingdom
SOURCE: Oncology Research, (1994) 6/10-11 (461-475).
ISSN: 0965-0407 CODEN: ONREE8
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The enzyme-directed approach to bioreductive drug development is designed to take advantage of the fact that the selectivity of bioreductive anticancer agents can be governed not only by the well-established difference in oxygen content of turnout vs. normal tissues, but also by the level of expression of enzymes catalyzing the reductive activation process. This can add value to bioreductive drug development in two ways. First, by using enzyme profiling to help guide the selection of patients most likely to respond to a particular bioreductive agent. And second, to aid the discovery of new and improved bioreductive drugs by optimising structure to suit the catalytic preferences of a given reductase enzyme. In this commentary, recent progress in the area of enzyme-directed bioreductive drug development is reviewed with emphasis on quinone anticancer agents and quinone reducing enzymes, particularly DT-diaphorase, which is often hyperexpressed in cancer tissue. The enzyme-directed approach has led to the development of the indoloquinone EO9, which is now in early clinical trials, and the diaziridinyl-benzoquinone methyl-DZQ, which has been selected very recently for clinical development. The complex interplay of the levels of oxygen and of DT-diaphorase governs the effectiveness of these agents and other quinones such as mitomycin C. A model is proposed to account for the behaviour observed. Advantages and disadvantages of the enzyme-directed bioreductive approach are summarised and future prospects are critically assessed.

L14 ANSWER 11 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94211045 EMBASE
DOCUMENT NUMBER: 1994211045
TITLE: Metabolism of bioreductive antitumor compounds by purified rat and **human** DT-diaphorases.
AUTHOR: Beall H.D.; Mulcahy R.T.; Siegel D.; Traver R.D.; Gibson N.W.; Ross D.
CORPORATE SOURCE: Univ. of Colorado School of Pharmacy, Box C238, 4200 E. Ninth Avenue,Denver, CO 80262, United States
SOURCE: Cancer Research, (1994) 54/12 (3196-3201).
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry

030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The metabolisms of two standard electron acceptors and a series of bioreductive antitumor compounds by purified rat and **human** DT-diaphorases (DTD) were compared. DTD was purified from rat liver cytosol and from Escherichia coli in which rat liver or **human** lung tumor DTD complementary DNA was expressed. K(m) and k(cat) values for menadione and 2,6- dichlorophenolindophenol reduction were similar for the three enzyme preparations except that rat E. coli DTD had 2-3-fold higher k(cat) values for both menadione and 2,6-dichlorophenolindophenol and a 2-3-fold higher K(m) for menadione than either rat liver or **human** E. coli DTD. Reduction of the antitumor compounds was 1.9-4.9 times faster with rat E. coli DTD than with **human** E. coli DTD. The antitumor compounds were reduced in the following order by rat E. coli DTD: 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone > streptonigrin > mitomycin A > diaziquone > mitomycin C (MC) > 5-(aziridin-1- yl)-2,4-dinitrobenzamide. The order was the same for **human** E. coli DTD with one exception; diaziquone was reduced slightly faster than mitomycin A. Metabolism of doxorubicin could not be detected using rat or **human** E. coli DTD. MC-induced DNA cross-linking was also more efficient using rat E. coli DTD relative to **human** E. coli DTD. Metabolism of MC by rat and **human** E. coli DTD was also compared under aerobic and hypoxic conditions. Rates of reduction of MC and metabolite formation were similar under aerobic and hypoxic conditions, and the toxicity of MC to DTD-rich HT-29 cells was also similar in aerobic and hypoxic conditions. In contrast, the toxicity of MC to DTD-deficient BE cells was potentiated markedly under hypoxia. These data show that although small catalytic differences between rat and **human** E. coli DTD can be observed, compounds such as 2,5-dimethyl-3,6-diaziridinyl-1,4- benzoquinone and streptonigrin are still excellent substrates for the **human** enzyme and may be useful in the therapy of tumors high in DTD activity. In addition, metabolism of MC by rat and **human** E. coli DTD was similar in aerobic and hypoxic conditions; in agreement with these data, cytotoxicity of MC to a DTD-rich cell line was oxygen independent. Increased MC cytotoxicity under hypoxia appears to be mediated by enzymes other than DTD.

L14 ANSWER 12 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93220695 EMBASE
DOCUMENT NUMBER: 1993220695
TITLE: The bioactivation of **CB 1954** and its use as a prodrug in antibody-directed enzyme prodrug therapy (ADEPT).
AUTHOR: Knox R.J.; Friedlos F.; Boland M.P.
CORPORATE SOURCE: Molecular Pharmacology Unit, Institute of Cancer Research, Cotswold Rd.,Sutton, Surrey SM2 5NG, United Kingdom
SOURCE: Cancer and Metastasis Reviews, (1993) 12/2 (195-212). ISSN: 0167-7659 CODEN: CMRED4
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Walker cells in vivo or in vitro are exceptionally sensitive to the monofunctional alkylating agent **CB 1954** (5-(aziridin-1-yl)-2,4- dinitrobenzamide). The basis of the sensitivity is that **CB 1954** forms DNA interstrand crosslinks in Walker cells but not in insensitive cells. Crosslink formation is due to the aerobic reduction of **CB 1954** to form 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide by the enzyme DT

diaphorase. The 4-hydroxylamine can not crosslink DNA directly but requires further activation by a non-enzymatic reaction with a thioester (such as acetyl coenzyme A). As predicted from their measured DT diaphorase activities, a number of rat hepatoma and hepatocyte cell lines are also sensitive to **CB 1954**. However, no **CB 1954**-sensitive tumours or cell lines of **human** origin have been found. This is because the rate of reduction of **CB 1954** by the **human** form of DT diaphorase is much lower than that of the Walker enzyme (ratio of k(cat) = 6.4). To overcome this intrinsic resistance of **human** cells towards **CB 1954** a number of strategies have been developed. First, analogues have been developed that are more rapidly reduced by the **human** form of **CB 1954**. Second, the cytotoxicity of **CB 1954** can be potentiated by reduced pyridinium compounds. Third, a **CB 1954** activating enzyme can be targeted to **human** tumours by conjugating it to an antibody (ADEPT). A nitroreductase enzyme has been isolated from E. coli that can bioactivate **CB 1954** much more rapidly than Walker DT diaphorase and is very suitable for ADEPT. Thus **CB 1954** may have a role in the therapy of **human** tumours.

L14 ANSWER 13 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93220689 EMBASE
DOCUMENT NUMBER: 1993220689
TITLE: NAD(P)H:Quinone oxidoreductase1 (DT-diaphorase) expression in normal and tumor tissues.
AUTHOR: Belinsky M.; Jaiswal A.K.
CORPORATE SOURCE: Dept. of Pharmacology, Fox Chase Cancer Center, 7701 Burholme Avenue,Philadelphia, PA 19111, United States
SOURCE: Cancer and Metastasis Reviews, (1993) 12/2 (103-117). ISSN: 0167-7659 CODEN: CMRED4
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB NAD(P)H:Quinone Oxidoreductase1 (NQO1) also known as DT-diaphorase is a flavoprotein that catalyzes the two-electron reduction of quinones, quinone imines and azo-dyes and thereby protects cells against mutagenicity and carcinogenicity resulting from free radicals and toxic oxygen metabolites generated by the one-electron reductions catalyzed by cytochromes P450 and other enzymes. High levels of NQO1 gene expression have been observed in liver, lung, colon and breast tumors as compared to normal tissues of the same origin. The transcription of the NQO1 gene is activated in response to exposure to bifunctional (e.g. β-naphthoflavone (β-NF), 2, 3, 7, 8 tetrachorodibenzo-p-dioxin (TCDD)) and monofunctional (phenolic antioxidants/chemoprotectors e.g. 2(3)-tert-butyl-4-hydroxy-anisole (BHA)) inducers. The high level of expression of the NQO1 gene and its induction by β-NF and BHA require the presence of an AP1 binding site contained within the **human** Antioxidant Response Element (hARE) and are mediated by products of proto-oncogenes, Jun and Fos. Induction of NQO1 gene expression involves transfer of a redox signal from xenobiotics to unknown 'redox protein(s)' which in turn, modify the Jun and Fos proteins for greater affinity towards the AP1 site of the NQO1 gene and activates transcription. The expression and regulation of the NQO1 gene is complex as many additional cis-elements have been identified in the promoter region and is a subject of great future interest. In addition to established tumors, NQO1 gene expression is also increased in developing tumors, indicating a role in cellular defense during tumorogenesis. It has been proposed that low molecular weight substance(s) can diffuse from tumor cells into surrounding normal cells and activate the expression of the NQO1 gene. Purification and characterization of such substance(s) may provide

important information in regard to the mechanism of activation of NQO1 gene expression and the role of increased NQO1 expression in tumor development. In view of the general consensus that NQO1 is over-expressed in tumor cells and the realization that NQO1 may either activate or detoxify xenobiotics, it is important to establish the role of NQO1 in the activation, and the detoxification of xenobiotics and drugs and in the intrinsic sensitivity of tumors to bioreductive alkylating aziridinyl benzoquinones such as diaziquone (AZQ), mitomycin C (MMC), and indoloquinone EO9, as well as to the dinitrophenyl aziridine, **CB1954**, and the benzotriazine-di-N-oxide, SR 4233.

L14 ANSWER 14 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92166714 EMBASE
DOCUMENT NUMBER: 1992166714
TITLE: Antibody-directed enzyme prodrug therapy (ADEPT) and its application to cancer treatment.
AUTHOR: Wilman D.E.V.
CORPORATE SOURCE: Drug Development Section, Institute of Cancer Research, Cancer Research Campaign Laboratory, Cotswold Road,Sutton SM2 5NG, United Kingdom
SOURCE: Current Opinion in Therapeutic Patents, (1992) 2/4 (364-373).
ISSN: 0962-2594 CODEN: COTPES
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English

L14 ANSWER 15 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92155318 EMBASE
DOCUMENT NUMBER: 1992155318
TITLE: The role of **human** and rodent DT-diaphorase in the reductive metabolism of hypoxic cell cytotoxins.
AUTHOR: Walton M.I.; Sugget N.; Workman P.
CORPORATE SOURCE: CRC Beatson Laboratories, CRC Department of Medical Oncology, University of Glasgow, Switchback Road,Bearsden, Glasgow G61 1BD, United Kingdom
SOURCE: International Journal of Radiation Oncology Biology Physics, (1992) 22/4 (643-647).
ISSN: 0360-3016 CODEN: IOBPD3
COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB DT-diaphorase is a unique two electron (2e) donating reductase catalyzing either bioactivation or bioprotection reactions. Using **human** and rodent DT- diaphorase preparations (cell extracts and purified enzyme) we have characterized the reductive metabolism of the hypoxic cell cytotoxins EO9, mitomycin C (MMC), **CB 1954**, and SR 4233 in vitro.
Drug metabolism was assayed spectrophotometrically or by HPLC, with dicoumarol as a selective inhibitor. DNA damage was measured using an agarose gel mobility technique with plasmid pBR322 DNA. The developmental indoloquinone, EO9, was metabolized by both rat Walker and **human** HT29 tumor DT-diaphorases. Reduction proceeded 5-fold more efficiently with the rat than the **human** tumor enzyme and resulted in single-strand breaks in plasmid DNA. The structurally related MMC was metabolized

much more slowly than EO9 by the rat Walker tumor enzyme and there was no detectable reaction with the **human** HT29 tumor DT-diaphorase. No DNA damage was seen with MMC for either enzyme. The **dinitrophenylaziridine CB 1954** was reduced by both **human** and rat enzymes forming, preferentially, the highly toxic 4-hydroxylamine as a 4e reduction product. Rates were 3-fold lower than for the **human** tumor enzyme. SR 4233 was also reduced by the rat tumor enzyme predominantly via 4e reduction to the benzotriazine SR 4330, in a novel reaction mechanism. This appears to be a bioprotection pathway that bypasses the toxic 1e radical formed by other reductases. Such information may be valuable in the selection of hypoxic cell cytoxins to treat **human** tumors high or low in DT-diaphorase and should facilitate 'enzyme-directed' analogue development.

L14 ANSWER 16 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92135372 EMBASE
DOCUMENT NUMBER: 1992135372
TITLE: DT-diapharose and cancer chemotherapy.
AUTHOR: Riley R.J.; Workman P.
CORPORATE SOURCE: Department of Medical Oncology, CRC Beatson Laboratories, University of Glasgow, Switchback Road,Bearsden G61 1BD, United Kingdom
SOURCE: Biochemical Pharmacology, (1992) 43/8 (1657-1669). ISSN: 0006-2952 CODEN: BCPCA6
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 016 Cancer
022 Human Genetics
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English

L14 ANSWER 17 OF 17 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 91108509 EMBASE
DOCUMENT NUMBER: 1991108509
TITLE: The differences in kinetics of rat and **human** DT diaphorase result in a differential sensitivity of derived cell lines to **CB 1954** (5-(aziridin-1-yl)-2,4-dinitrobenzamide).
AUTHOR: Boland M.P.; Knox R.J.; Roberts J.J.
CORPORATE SOURCE: Molecular Pharmacology Unit, Section of Drug Development, Institute of Cancer Research, Cotswold Road,Sutton, Surrey SM2 5NG, United Kingdom
SOURCE: Biochemical Pharmacology, (1991) 41/6-7 (867-875). ISSN: 0006-2952 CODEN: BCPCA6
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB DT diaphorase (NAD(P)H dehydrogenase (quinone), EC 1.6.99.2) isolated from Walker 256 rat carcinoma cells can convert **CB 1954** (5-(aziridin-1-yl)2,4-dinitrobenzamide) to a cytotoxic DNA interstrand cross-linking agent. This is achieved by reduction of the 4-nitro group of **CB 1954** to produce the hydroxylamino species, a bioactivation which accounts for the much greater sensitivitiy of Walker cells to **CB 1954** when compared with other cells which are unable to carry out this reduction (Knox et al., Biochem Pharmacol 37: 4661-4669 and 4671-4677, 1988). As predicted from their measured DT diaphorase activities a number of rat hepatoma and hepatocyte cell lines were also shown to be sensitive to **CB 1954**. However,

no **CB 1954**-sensitive cell lines of **human** origin were found, although levels of DT diaphorase similar to those in the sensitive rat cells were present in these cells. The **human** cells were assensitive as rat cells to the active form of **CB 1954** (5-(aziridin-1-yl)-4-hydroxylamoni-2-nitrobenzamide). DT diaphorase, purified to homogeneity from **human** Hep G2 cells, did metabolize **CB 1954** to this 4-hydroxylamino product, but the rate of **CB 1954** reduction and thus production of the cytotoxic product, was much lower than that of purified Walker enzyme (ratio of K(cat) = 6.4). In addition, **CB 1954** could be considered an inhibitor of, rather than a substrate for, the **human** form of DT diaphorase. The purified rat and **human** DT diaphorases possessed otherwise similar biochemical and molecular properties. These findings explain the decreased sensitivity towards **CB 1954** of **human** cell lines when compared to rat cell lines.

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=> d his

     (FILE 'HOME' ENTERED AT 17:21:55 ON 19 NOV 2002)

     FILE 'MEDLINE, CANCERLIT, BIOSIS, EMBASE, SCISEARCH' ENTERED AT 17:22:02
     ON 19 NOV 2002
L1          271 S DINITROPHENYLAZIRIDINE OR CB1954
L2          619 S DINITROPHENYLAZIRIDINE OR CB1954 OR CB-1954
L3          123 S L2 AND VIVO
L4           48 DUP REM L3 (75 DUPLICATES REMOVED)
L5           26 S L4 AND HUMAN
L6            9 S L5 AND PY<=1997
L7          273 S L2 AND (REDUC? OR NICOT?)
L8          167 S L7 AND PY<=1997
L9           68 DUP REM L8 (99 DUPLICATES REMOVED)
L10          26 S L9 AND HUMAN
L11          73 S L2 AND NICOT?
L12          47 S L11 AND HUMAN
L13          28 DUP REM L12 (19 DUPLICATES REMOVED)
L14          17 S L13 AND PY<=1997

=> s l14 and vivo
L15           1 L14 AND VIVO

=> d ibib abs 1

L15  ANSWER 1 OF 1  EMBASE  COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER:   93220695  EMBASE
DOCUMENT NUMBER:    1993220695
TITLE:              The bioactivation of CB 1954 and its
                    use as a prodrug in antibody-directed enzyme prodrug
                    therapy (ADEPT).
AUTHOR:             Knox R.J.; Friedlos F.; Boland M.P.
CORPORATE SOURCE:   Molecular Pharmacology Unit, Institute of Cancer Research,
                    Cotswold Rd.,Sutton, Surrey SM2 5NG, United Kingdom
SOURCE:             Cancer and Metastasis Reviews, (1993) 12/2 (195-212).
                    ISSN: 0167-7659  CODEN: CMRED4
COUNTRY:            United States
DOCUMENT TYPE:      Journal; General Review
FILE SEGMENT:       016     Cancer
                    030     Pharmacology
                    037     Drug Literature Index
LANGUAGE:           English
SUMMARY LANGUAGE:   English
AB   Walker cells in vivo or in vitro are exceptionally sensitive to
     the monofunctional alkylating agent CB 1954
```

(5-(aziridin-1-yl)-2,4- dinitrobenzamide). The basis of the sensitivity is that **CB 1954** forms DNA interstrand crosslinks in Walker cells but not in insensitive cells. Crosslink formation is due to the aerobic reduction of **CB 1954** to form 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide by the enzyme DT diaphorase. The 4-hydroxylamine can not crosslink DNA directly but requires further activation by a non-enzymatic reaction with a thioester (such as acetyl coenzyme A). As predicted from their measured DT diaphorase activities, a number of rat hepatoma and hepatocyte cell lines are also sensitive to **CB 1954**. However, no **CB 1954**-sensitive tumours or cell lines of **human** origin have been found. This is because the rate of reduction of **CB 1954** by the **human** form of DT diaphorase is much lower than that of the Walker enzyme (ratio of k(cat) = 6.4). To overcome this intrinsic resistance of **human** cells towards **CB 1954** a number of strategies have been developed. First, analogues have been developed that are more rapidly reduced by the **human** form of **CB 1954**. Second, the cytotoxicity of **CB 1954** can be potentiated by reduced pyridinium compounds. Third, a **CB 1954** activating enzyme can be targeted to **human** tumours by conjugating it to an antibody (ADEPT). A nitroreductase enzyme has been isolated from E. coli that can bioactivate **CB 1954** much more rapidly than Walker DT diaphorase and is very suitable for ADEPT. Thus **CB 1954** may have a role in the therapy of **human** tumours.

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